MOLYBDENUM—IS IT AN ESSENTIAL TRACE METAL?*

NAJI N. ABUMRAD, M.D.

Department of Surgery
Vanderbilt University Medical Center
Nashville, Tennessee

BIOCHEMICAL BASIS OF ESSENTIALITY

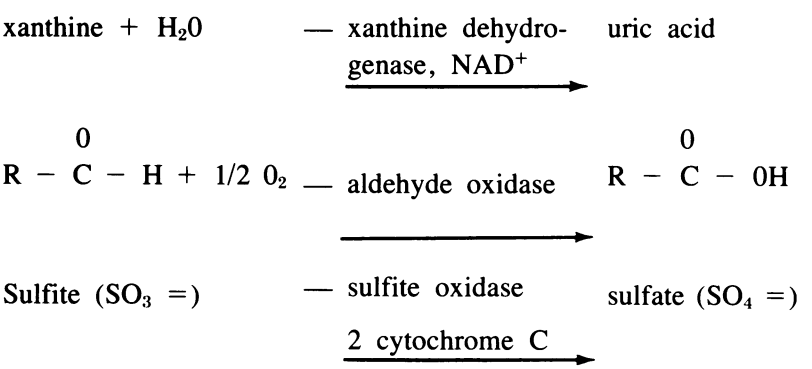
MOLYBDENUM, with an atomic number of 42, is presently considered an essential trace element that participates in a number of enzymatic reactions in many microorganisms and mammals. There are several known molybdenum-containing enzymes: xanthine oxidase/dehydrogenase, aldehyde oxidase, and sulfite oxidase. Moreover, molybdenum model systems have been reported to catalyze the reduction of nitrate, either for the assimilation of nitrogen or for oxidation, and to reduce nitrogen (N_2), attesting to the important role this metal plays in the earth's nitrogen cycle.[1] The common feature of all these reactions is transfer of an even number of electrons to or from substrates. They catalyze the transfer of an oxygen atom from water to a wide variety of compounds.

The aldehyde oxidases oxidize and detoxify various aldehydes, pyrimidines, purines, pteridines, and related compounds. Xanthine oxidase catalyzes the transformation of xanthine to uric acid. In addition, hypoxanthine and other relative compounds are also oxidized by this enzyme. Xanthine dehydrogenases behave similarly, transferring electrons, however, to receptors other than oxygen.[2] Because of the structural and functional similarities between the aldehyde and xanthine oxidases, it was suggested that these two cytoplasmic enzyme complexes arose from a common ancestral protein.[3] Sulfite oxidase, an intramitochondrial enzyme found in a variety of organisms, catalyzes the transformation of sulfite to sulfate.

$$\text{xanthine, hypoxanthine, others} + 1/2\ O_2 \xrightarrow{\text{xanthine oxidase}} \text{uric acid}$$

*Presented as part of the *Working Conference on Parenteral Trace Elements-II* held by the Section on Clinical Nutrition of the New York Academy of Medicine and the Department of Foods and Nutrition of the American Medical Association at the Academy September 14 and 15, 1982.

Address for reprint requests: Department of Surgery, Vanderbilt University Medical Center, Nashville, TN 37232



Dietary Requirements

Studies by Tipton, Stewart, and Dickson estimated that the average Western diet contains 200 to 500 μg of molybdenum per day.[4] It is estimated that children and preadolescents require more molybdenum than adults.[5] Studies using goats[6] and chicks[7] have suggested that prolonged exposure to low molybdenum diets results in reduced maternal to fetal transfer of the element. As a result, offspring suffer poor growth patterns and high mortality rates. Inclusion of as little as 1 to 2 ppm of molybdenum in the diets of the mother[7] or offspring[6] resulted in significant enhancement of offspring growth and survival.

Absorption and Metabolism

After rapid absorption from the gastrointestinal tract, molybdenum is transported to the liver by the blood, expecially the erythrocytes.[8] The liver extracts molybdenum with the excess excreted in bile, thus establishing an enterohepatic cycle. In human autopsy studies, the greatest amounts of molybdenum occurred in the liver, kidneys, small intestinal mucosa, and adrenals.[9] Molybdenum is primarily excreted in the urine and to some extent in the bile. Normal fecal losses amount to only a third of urinary losses.

In certain gastrointestinal disorders, however, extensive fecal losses may occur. Balance data obtained in our laboratory from two subjects with Crohn's enterocolitis demonstrated that during active disease, gastrointestinal losses of molybdenum may exceed 400 μg/day.

Evidence for Human Requirements

Despite thorough investigation of the molybdenum-containing enzymes,

the essentiality of this trace element has been very difficult to establish. There are few instances in which a dietary deficiency of this element has been directly implicated as a cause of poor growth and fewer in which it has been recognized to be the cause of metabolic defects provoking the appearance of characteristic pathological lesions. Early investigations revealed that when rats and chicks were fed a molybdenum-deficient diet, substantial decreases in the activity of intestinal oxidase resulted, while effects on liver xanthine oxidase were either very small or absent.[10,11] Only when tungsten, a competitive inhibitor to molybdenum, was included in the diet, was there a substantial decline in both liver and intestinal xanthine oxidase activities.[10]

We have recently reported[12] a case of a 24-year-old Caucasian man who underwent multiple intestinal resections since the age of 10 years and required total parenteral nutrition for permanent support. The first six months were quite uneventful; however, subsequently he developed multiple episodes of a syndrome characterized by tachycardia, tachypnea, severe bifrontal headache, night blindness, nausea, vomiting, central scotomas, lethargy, disorientation, and ultimately coma. The biochemical abnormalities (shown in Tables I and II) were characterized by very high levels of plasma methionine and almost undetectable levels of serum uric acid. Balance studies demonstrated that sulfate excretions accounted for less than 40% of the ingested sulfur load as compared to controls of 80%. Most of the sulfur load remained either as sulfite in the blood or was excreted as thiosulfate ($\sim$ 50% versus 2% in the control group) as shown in Table II. These abnormalities indicated that there was a block in the oxidation of sulfite to sulfate. Moreover, the low urinary and serum uric acids were associated with excessive excretion of the oxypurines, hypoxanthine and xanthine, suggesting that there was a similar block in the transformation of xanthine and hypoxanthine to uric acid.

Supplementation of total parenteral nutrition solutions with ammonium molybdate (300 μg/day) reversed both the clinical and biochemical abnormalities as shown in Tables I and II. The excretion of urinary sulfate improved almost to normal levels, while serum uric acid and urinary excretion of uric acid increased, with a concomitant decrease in the urinary excretion of the oxypurines.

This human study[12] and many animal studies have given no proof that the observed deleterious effects on growth and morbidity are attributable to xanthine oxidase deficiency.[13] Instead, such changes have been attributed to a deficiency in sulfite oxidase enzymes.[14,15] Whether the enzyme

TABLE I. PLASMA METHIONINE LEVELS AND URINARY SULFUR METABOLITE EXCRETION BEFORE AND AFTER AMMONIUM MOLYBDATE THERAPY*

	Before	*After*	
	Ammonium molybdate		*Control†*
Plasma methionine (μmol/L)	123	73	18
Total sulfur excretion (mM/d)	14.8	12.5	24 ± 1.2
Inorganic sulfate excretion (mM/d)	5.5	8.4	183 ± 1.2
Thiosulfate excretion (mM/d)	7.0	0.9	0.4 ± 0.08
Sulfite excretion (qualitative)**	+ + +	—	—

*Balance studies were performed with the patient and control subjects receiving equimolar amounts of total parenteral nutrition solutions containing L-methionine (22.7 mM/d) as the only source of sulfur-containing amino acids.
**Sulfite excretion was assayed qualitatively, using sulfite screening strips (Machery, Nagel and Co., Germany).
†Three surgical subjects were used as controls requiring total parenteral nutrition therapy for a minimum of three weeks. Values are expressed mean ± SEM.

TABLE II. SERUM URIC ACID LEVELS AND URINARY URIC ACID AND OXYPURINE EXCRETION BEFORE AND AFTER AMMONIUM MOLYBDATE THERAPY*

	Before	*After*	
	Ammonium molybdate		*Control†*
Serum Uric Acid (mg/dl)	0.8	5.7	4.8 ± 1.1
Uric acid excretion (mg/d)	115	540	475 ± 54
Urinary hypoxanthine excretion (mg/d)	380	75	< 50
Urinary xanthine excretion (mg/d)	1,600	140	< 50

*Experimental conditions are the same as in Table I.

functions to detoxify sulfite to provide the essential sulfate ions remains to be determined. When our subject[12] received hydrogen bisulfite (1.8 g/day) intravenously, an amount equivalent to that present in 3 L of commercially available total parenteral nutrition solutions, similar biochemical and clinical abnormalities became apparent by the end of four days. Plasma

methionine increased by 2.4-fold, and the patient developed mental confusion, necessitating withdrawal of the infusion. These cumulative observations attest to the protective role of sulfite oxidase enzyme against abnormal levels of sulfite. These observations are consistent with data obtained in a rat model of sulfite deficiency, maintained on low molybdenum diet enriched with tungsten. Animals depleted of tissue sulfite oxidase activity were significantly more susceptible to toxicity from injected or respired sulfite.[15]

Since direct measurements of tissue molybdenum levels or enzyme activities were not obtained in our reported case,[6] convincing evidence for the essentiality of this trace element in humans will remain unanswerable at the present time. Balance studies were recently carried out in our laboratory involving two subjects with granulomatous Crohn's enterocolitis who were maintained on prolonged total parenteral nutrition therapy:

Case 1. Balance studies were performed on a 38-year-old white woman with known history of active recurrent Crohn's disease of the ileum who was maintained on prolonged total parenteral nutrition therapy. She ultimately required resection of her distal ileum. Urine and ileostomy fluids were collected preoperatively and for 15 days postoperatively. As shown in Figure 1, prior to surgery there was a tremendous output of molybdenum from the ileostomy, averaging approximately 530 μg/day, while her urinary output was 75 to 100 μg/day. Postoperatively, the ileostomy output of molybdenum decreased, while urinary molybdenum output was minimally increased.

Case 2. A 27-year-old white man presented with known recurrence of Crohn's terminal ileitis. Previous history included multiple small and large bowel resections with creation of an ileostomy. Balance studies were performed while he was on maintenance total parenteral nutrition solutions (2,500-3,000 calories per day) and are shown in Figure 2. Ileostomy output of molybdenum was 300-350 μg/day, while the urinary output was negligible. Plasma uric acid level was low, with associated reduction in urinary uric acid output. Plasma molybdate levels were within normal limits. Supplementation of parenteral nutrition solutions with 500 μg/day of ammonium molybdate resulted in an increase in urinary uric acid output and a significant increase in plasma uric acid. No change was observed in either plasma molybdate levels or in molybdenum output via the ileostomy.

The human studies indicates that extensive losses of molybdenum from the gastrointestinal tract could occur in patients with Crohn's disease.

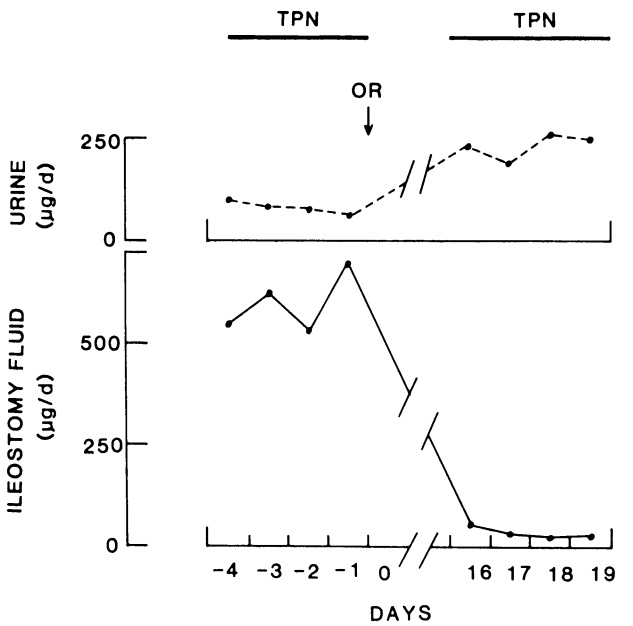


Fig. 1. Daily urinary and ileostomy fluid content in a 38-year-old woman with active Crohn's disease

Hence, it is possible to speculate that such losses would result in true molybdenum deficiency in the tissues with associated decrease in both xanthine oxidase and sulfite oxidase activities, thus resulting in the clinical and biochemical syndrome described.[12]

Molybdenum Content in TPN Solutions

The molybdenum content of total parenteral nutrition solutions is unknown.

Toxicity

Molybdenum has a very low order of toxicity,[17] and animal studies have established that toxicity depends on the compound utilized. The soluble hexavalent form produces the least toxic effects, while molybdenum trioxide produces the most.[17] Cattle fed on pastures with high molybdenum content in the soil suffered high mortality unless removed from the area of grazing. The diarrhea, anemia, and defective melanogenesis ob-

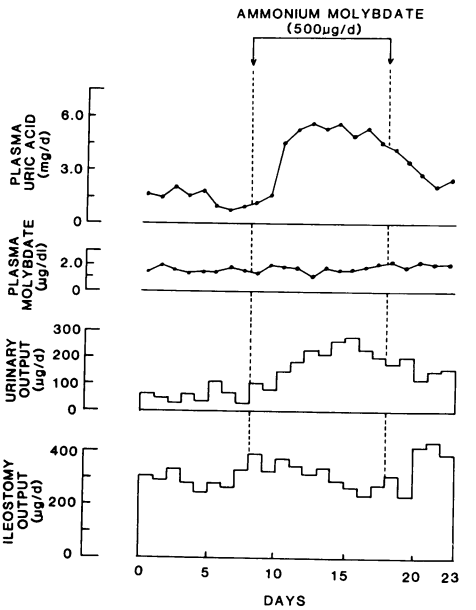


Fig. 2. Effect of ammonium molybdate infusion on plasma uric acid, plasma molybdate and urinary and ileostomy molybdenate output in a 27-year-old male with Crohn's ileitis

served in affected animals were found to be caused by severe copper deficiency.[18] Conversely, rat studies have shown that molybdenum promoted hepatic copper retention.[19] Moreover, studies by Gray and Daniel[20] in rats have established a role for sulfide in the molybdenum-copper antagonism. They found that a high sulfur-containing diet, in the presence of adequate amounts of molybdenum, results in a severe hepatic copper deficiency, associated with severe diarrhea, anemia, and a high mortality rate.[20] These observations lend further complexity to this subject, emphasizing the need for more investigations in this area, especially as it pertains to the use of total parenteral nutrition solutions in humans.

Summary

Many of the observations reported here were made at a time when the requirement of the trace elements were not so well defined as at present. Many of these studies were hampered by inadequate methods to measure molybdate concentration in tussues and body fluid compartments. The

recent advent of better techniques, such as the neutron activation analysis[21] and electron emission spectroscopy,[8] provides a strong case for reinvestigating the essentiality and requirements of this trace element.

ACKNOWLEDGEMENTS

I thank Rose Hornsby for her technical assistance and Tom Ebers for his efforts in the final preparation of the manuscript.

REFERENCES

1. Moura, J. J. G. and Xavier, A. V.: Molybdenum in Proteins. In: *New Trends in Bioinorganic Chemistry,* Williams, R. J. P. and DeSilva, J. J. R. F., editors. New York, Academic, 1978, pp. 79-119.
2. Bray, R. C.: Molybdenum iron-sulfur flavin hydroxylases and related enzymes. *Enzymes 12:* 299-419, 1975.
3. Krenitsky, T. A., Tuttle, J. V., Cattau, E. L., et al.: A comparison of the distribution and electron acceptor specificities of xanthine oxidase and aldehyde oxidase. *Comp. Biochem. Physiol. 49B:* 687-703, 1974.
4. Tipton, I. H., Stewart, P. L., and Dickson, J.: Patterns of elemental excretion in long term balance studies. *Health Phys. 16:* 455-62, 1969.
5. Engel, R. W., Price, N. O., and Miller, R. F.: Copper, manganese, cobalt and molybdenum balance in pre-adolescent girls. *J. Nutr. 92:* 197-204, 1967.
6. Anke, M., Grun, M., Partschfeld, M., et al.: Molybdenum Deficiency in Ruminants. In: *Trace Element Metabolism in Man and Animal,* Kirchgessner, M., editor. Berlin, Friesing Weihenstephan, 1978, vol. 3, pp. 230-33.
7. Payne, G. C.: Molybdenum Response Syndromes in Poultry. In: *Trace Element Metabolism in Man and Animal,* Kirchgessner, M., editor. Berlin, Friesing Weihenstephan, 1978, vol. 3, pp. 315-22.
8. Baert, N., Cornelius, R., and Hoste, J.: Molybdenum in human blood. *Clin. Chim. Acta. 68:* 355-56, 1976.
9. Underwood, E. J.: *Trace Element Metabolism in Animals.* New York, Academic, 1977, pp. 84-101.
10. Reichart, D. A. and Westerfield, W. W.: Isolation and identification of the xanthine oxidase factor as molybdenum. *J. Biol. Chem. 203:* 915-23, 1953.
11. Reid, B. L., Kurnick, A. A., Burroughs, R. N., et al.: Molybdenum in poultry nutrition. *Proc. Soc. Exp. Biol. Med. 94:* 737-40, 1957.
12. Abumrad, N. N., Schneider, A. J., Steel, D., and Rogers, L. S.: Amino acid intolerance during prolonged total parenteral nutrition reversed by molybdate therapy. *Am. J. Clin. Nutr. 34:* 2551-59, 1981.
13. Higgins, E. S., Richert, D. A., and Westerfeld, W. W.: Molybdenum deficiency and tungstate inhibition studies. *J. Nutr. 59:* 539-59, 1956.
14. Mudd, S. H., Irrevere, F., and Lasler, L.: Sulfite oxidase deficiency in man. Demonstration of the enzymic defect. *Science 156:* 1599-1602, 1967.
15. Cohen, H. J., Drew, R. T., Johnson, J. L., and Rajagopalan, K. V.: Molecular basis of the biological function of molybdenum: the relationship between sulfite oxidase and the acute toxicity of bisulfite and SO_2. *Proc. Nat. Acad. Sci.* (USA) *70:* 3655-59, 1973.
16. Johnson, J. L., Rajagopalan, K. V., and Cohen, H. J.: Molecular basis of the biological function of molybdenum. Effect of tungsten on xanthine oxidase and sulfite oxidase in the rat. *J. Biol. Chem. 249:* 859-66, 1974.
17. Mills, C. F., Monty, K. J., Ichihara, A., et al.: Metabolic effects of molyb-

denum toxicity in the rat. *J. Nutr. 65:* 129-42, 1958.
18. Fergusson, W. S., Lewis, A. H., and Watson, S. J.: The teart pastures of Somerset. I. The cause and cure of teartness. *J. Agric. Sci. Camb. 33:* 44-51, 1943.
19. Mills, C. F. and Mitchell, R. L.: Copper and molybdenum in subcellular fractions of rat liver. *Br. J. Nutr. 26:* 117-21, 1971.
20. Gray, L. F. and Daniel, L. J.: Effect of the copper status on the copper-molybdenum-sulphate interaction. *J. Nutr. 84:* 31-37, 1964.
21. Versieck, J., Hoste, J., Barbier, F., et al.: Determination of molybdenum in human serum by neutron activation analysis. *Clin. Chem. Acta 87:* 135-40, 1978.